

T H E S I S

On

A BACTERIOLOGICAL STUDY OF CERTAIN ORGANISMS
CAUSING THE SPOILAGE IN CANNED PEAS.

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A BACTERIOLOGICAL STUDY OF CERTAIN ORGANISMS
CAUSING THE SPOILAGE IN CANNED PEAS.

Introduction

Canning, as Bitting defined, (7) is "the art of preserving a food in a hermetically sealed container, the preservation being accomplished through sterilization by means of heat." The practice of canning is popularly employed throughout the civilized world as one of the best methods of food preservation from the standpoint of economics and hygiene. It affords the means of having wholesome, succulent vegetables or other products at all times and in places where the cost, or the labor of preparation would be prohibitive. As a result, the output of the canning factories in this country has increased many times during the last decade. Consequently, the question of spoilage occurring in canned food products has become of vastly more importance than ever before.

The problems of spoilage in canned products are very complicated. Much time has been devoted by early investigators with the view to enriching our knowledge of the cause or causes that bring about the decomposition of the canned products, and render their value as edibles worthless. Much light has been thrown upon these problems and many improvements have been accomplished in the canning industry during the recent years.

The scope of the canning industry is broad. The success of these investigations does not mean that all difficulties in the canning practice have been overcome. Each and every kind of product preserved to-day has its many unsolved problems. Therefore it is clear that further investigations in the field will be of inestimable value in preventing many unnecessary losses through the spoilage of canned goods.

An historical survey of the literature:

There have been many valuable articles relative to the bacteriology of the canning industry written during the past decade, but few are confined to any particular field of the canning industry. In order to bring to mind the progress that has been made regarding the bacteriology of canned goods, the writer was obliged to make an extensive bibliography of the subject so as to shed light upon this investigation.

Pasteur (22) on April 7, 1864 delivered his famous lecture at the Sorbonne. He showed that "If any solution containing organic matter is heated long enough, and protected from the micro-organisms in the air, it will remain unaltered." He held to the argument that "life comes from life but not from the dead matter," as was contended by many believers of the "Spontaneous Generation Theory of Life."

In 1895, Russell (23) brought forth the first re-

port on spoilage in canned goods based on bacteriological examination: This was in connection with the occurrence of "swells" in the pea canneries of Wisconsin. In his experiments, he succeeded in isolating two species of bacilli from the swelled cans of peas; however, the organisms found were neither described nor determined. He concluded that most of the troubles encountered are probably due to insufficient sterilization. Therefore he recommended with peas, which are naturally high in sugar content, that the temperature should be raised to 242 degrees F. and the pressure to 15 pounds for 28 minutes, but with those peas that are somewhat deficient in sugar content the temperature and the pressure recommended are the same as above, but he contended the time of processing should be increased to 30 minutes.

Hall (19) examined an outbreak of spoilage in canned peas. He found that the germ or the spore-forming bacillus identified as causing the spoilage, could survive when the cans were heated to 230 degrees F. for 30 minutes. He undertook an experiment in which he varied the temperatures and the time of heating and found that canned peas processed at 240 degrees F. for 30 minutes eliminated practically all swelled cans.

In 1917, the Department of Agriculture (26) published an article on "Canned Food Safe." They claimed that there was no danger from food poisoning. The type of food

poisoning known as botulism will not result from eating fruits or vegetables which have been canned by any of the methods recommended by the Department, provided directions have been carefully followed. It was also stated that since the spores of the *B. botulinus* are killed by one hour of heating at 175 degrees F., there is no reason to believe that the botulism organism will survive such treatment. However, a warning was given against eating foods showing any sign of spoilage.

Dickson (14) stated in his article on "Botulism: ---"1917, that during his investigation on the occurrence of botulism on the Pacific Coast, he had isolated *B. botulinus* from food in three cases. He then inoculated sterile canned peas, beans, and corn with *B. botulinus*, heating them according to the directions recommended by the government bacteriologists. Gas was produced within three weeks. *B. botulinus* and *B. subtilis* were recovered from the cans. Therefore he concluded that the "cold-pack method" as recommended is inadequate to kill "*B. botulinus*" spores if they happen to be present.

In 1918, he published another article (15) showing that botulism is not essentially a meat poison, but may also occur in canned vegetables and fruits. His experiment on infusion of canned green peas shows that *B. botulinus* will grow readily and will produce its toxin in a medium prepared from green peas. He concluded that

the methods usually employed in the home-canning vegetables and fruits are unsafe, and recommended that all home-canned vegetables should be cooked before they are eaten.

Duckwall (16) in 1905, published a book on canning and preserving of food products. He attempted to apply bacteriology to the whole canning industry. He devoted one chapter each on peas, tomatoes, and corn, in which he discussed their history, their composition and food value, methods of canning, and bacteria associated with spoilage. He isolated, identified and described the following organisms:- lactic acid bacteria, "*B. butyricus*", "*B. mesentericus vulgatus*", a butyric acid bacillus which was a strict anaerobe with terminal spores similar to "*B. tetani*", "*B. megatherium*", "*B. prodigiousus*", "*B. subtilis*", "*B. mesentericus ruber*", "*B. mycoides*" and some organisms not named but described. He recommended better facilities for handling the crop gathered from the field, and longer processing period at a higher temperature for canned goods.

Duckwall also claimed that the gas in cans with swelled ends has not necessarily arisen through the activity of micro-organisms, although undoubtedly they are by far the most frequent agent. Inadequate processing of fruits and vegetables may leave the seeds, if present, or some of the cells in a living condition, the result being that gases are given off through the respiration

of the cells. Swelled goods in which there is no evidence of contamination may sometimes be accounted for in this way.

Bigelow (5), chief chemist for the National Canners' Association Laboratory, emphasizes careful examination of both the external and the internal appearances of the cans in connection with the inspection of canned foods when it is brought under investigation. He contends that with non-acid foods such as peas, corn, etc., "swells" are usually due to decomposition while spoilage rarely occurred with acid fruits unless the can is leaky. In the absence of leaks, swelling is almost invariably due to hydrogen set free by the action of fruit acid on the metal of the containers.

Jordan (20), after examining a number of spoiled canned foods, stated that actual discovering of pathogenic bacteria in foods is a rare occurrence and in particular cases may have little or no practical significance. This might be anticipated because comparatively few bacteria of pathogenic significance are spore-formers.

Goss (18) found that the solution of tin by canned foods is neither dependent nor proportionate to the acidity alone but that in the foods of relatively low acidity, which dissolve large amounts of tin, the greater part of it is in the form of an insoluble and stable complex. He states that albumins, globulins and other proteins are negative colloids and are precipitated by an ion of

opposite charge from heavy metals such as tin.

Billings (6) declares that gas in "Swells" arises chiefly as a decomposition product of the activity of bacteria and yeasts. The presence of an excessive number of micro-organisms, dead or alive, in a canned product as he assumed may be explained in the following ways: First, the contents may have been partially decomposed by bacteria, yeasts and molds before being canned. The organisms are killed by processing. Secondly, swells may be due to failure of the processing in rendering a sterile product. Fermentation of some sort would naturally occur in these cans. Third, the fermenting organisms may reach the contents of a can through some minute openings due to defective soldering or a defective can, but which appear perfectly good cans to ordinary observation.

Ruth Normington (21) studied the heat resistant organisms from "cold-packed" canned peas. All organisms isolated were spore-forming bacilli. Among them, eight were gas-producers in sterile peas but not in other media. She demonstrated that "*B. subtilis*", "*B. ramosus*", "*B. ruber*", "*B. prodigiosus*", and "*B. viscosus*" will produce gas in peas but not in other media. From her findings, she held that pressure cooking is the only means of reducing the percentage of spoilage.

Georgia Burke (10) concluded that the toxin produced by "*B. botulinus*" will be entirely destroyed by boiling for five minutes, but the spores of this organism

could not be killed in jars of fruit by boiling less than five hours. She recommended that the best means of avoiding spoilage of canned goods due to the activity of such organisms are: First, to prevent spores of these organisms from entering the jars; second, that pressure cooking is the final and effective method of controlling this organism.

Weinzirl (27) examined 1018 samples of canned goods consisting of spoiled, suspected, underprocessed and market samples. He isolated 392 cultures of bacteria representing 38 species, and many varieties of yeasts and molds. The most prevalent species of bacteria were "*B. mesentericus*", "*B. subtilis*", "*B. thermoindifferens*", "*B. vulgatus*" and "*B. cereus*". He claimed that commercial canned foods as found in the markets are not always sterile, but may contain viable sporeforming bacteria. These bacteria were unable to grow due to the absence of oxygen. He concluded that a vacuum is essential to the preservation of canned foods under present methods of processing. He concluded that food poisoning organisms such as "*B. botulinus*" and "*B. enteritidis*" are not often found in commercial canned foods.

Sears and Putnan (24) present a new explanation in which the gaseous fermentation is produced by two organisms growing in symbiosis in a medium from which neither organism acting alone can produce gas. They conclude that

this phenomenon is of common occurrence. They explain this phenomenon by deducing that a combination of an acid former, capable of fermenting the given sugar, growing with a non-fermenting organism, may be expected to give gas. They explain that many cases of gas formation in canned foods may be due to symbiotic reaction of this kind.

Object of this investigation:

The growth of the canning industry makes the question of spoilage in canned goods a problem of monetary significance. With the view of throwing light on some of these perplexing problems, the writer has undertaken the following investigation. The method of procedure has been to isolate and identify the organisms, to determine their "Thermal Death Point", and to determine the gas fermentation from the organisms described.

Procedure:

During the Fall and Winter terms of the College Year 1923-24, twenty-six cans of spoiled and suspected canned peas were gathered from grocery stores. Five of them were probably leaky, because the seams had become very rusty. The remainder may or may not have been leaking,--this could not entirely be determined due to lack of any noticeable evidence of leakage on the cans, but all these cans collected were "swells". Twelve commercial cans of peas were purchased and used as a checking

purpose and for making special media. The usual bacteriological methods and technique were employed in this work.

1. Determination of leakage in cans,--the task of determining whether a can is leaking or non-leaking is not simple. There is no accurate method for making this determination. A careful inspection, however, given to the suspected cans before passing judgment on them will serve our purpose. In determining the leakage in cans, classification may be made under three heads as follows: (a) Cans showing evident leakage--for example, pinholes and openings readily seen by the naked eye; (b) Cans which may leak but not evidently so; and (c) Cans which are perfectly sealed.

2. Determination of swelled cans,--According to Dr. W. D. Bigelow (4) swelled cans may be classified as follows: (a) "Soft swelled" cans showing bulging of the ends but yielding readily under pressure of the thumb; (b) "Hard swells", yielding only slightly or not at all under pressure of the thumb; and (c) "Buckled cans" showing straightening of the seam. "Soft swells" may be due to overfilling, high temperature, action of acid on metal or insufficient vacuum; whereas, both the "hard swells" and the "buckled cans" are usually due to decomposition through the activity of organisms unless in case of the strongly acid fruits.

3. Culture media employed in the isolation work,--
In order to be able to isolate the organisms growing in

the canned peas, it is necessary to use several kinds of media. The ones used were:

- (a) Plain nutrient agar plates at 37 degrees C. were used to isolate organisms which grew best at blood heat, and at 50 degrees C. for searching for thermophiles.
- (b) Dextrose agar plates were used to favor yeasts and other organisms which do not grow well on nutrient agar.
- (c) Deep dextrose shake cultures were made to serve in isolating anaerobes.
- (d) Special media such as pea-agar, - This is essential because some organisms will thrive only when certain required food substances are present in the media.
- (e) Lactose and pea fermentation tubes were used to detect the gas-producing organisms.

All media used were made from nutrient broth having three grams of beef extract and five grams of peptone to one liter of water. Plain nutrient agar is prepared by adding 1.5% of agar agar to every liter of plain nutrient broth, while sugar agar or broth may be made by dissolving 1% of the sugar in the melted agar or broth. The pea-agar or -broth was made by adding 500 cc. pea juice to 500 cc. of melted agar or broth respectively.

4. Size of sample added to media, - A varying portion from three loopfuls to one cubic centimeter were used for inoculation. It is important to remember that in many cases, where too small a sample was used organisms may be missed, whereas, on the other hand, if too large, there is a possibility of outgrowth by one predominating

species when several are present. However, by using varying portions and by employing several plates in each test pure cultures could be isolated.

5. Identification of organisms isolated,- The organisms isolated from the spoiled canned peas were bacteria, yeasts and molds. Bacteria of several species were present in all cans, while yeasts and molds were isolated from cans that had produced evident leakage, and from a few others that were suspected to be leaking. It is evident, that the presence of yeasts and molds in canned peas indicates either that the peas have been canned in defective cans or that they have been underprocessed--because neither yeasts nor molds are able to survive when foods are heated to 170 degrees F. or over. Consequently, no attempt was made to classify the yeasts and molds isolated. Only the bacteria that are capable of withstanding high processing temperatures and those that possess important bearings on spoilage in the canned peas were studied.

Bacteriological analysis of canned peas:

The spoiled or suspected cans of peas were studied according to the following outline and the results tabulated in Table I.

Of twenty-six cans of peas analyzed, five showed evident leakage, six doubtful, and fifteen no apparent

leakage. They were all "swells". In all, two could be considered as "springers"- because we could find no evidence of spoilage among them with exception of slightly bulged ends. The odor, color, and condition of the peas and their liquids were normal. Bacteriological methods failed to show the presence of bacteria. The contents of the remaining cans, twenty-four in number, were more or less decomposed, and organisms were successfully isolated from them with three exceptions.

Of these twenty-four cans containing organisms, six (see foot note in Table I.) were evidently from the same pack. Examination of these disclosed bacillus organisms apparently of the same species. These organisms were readily stained with carbol-fuchsin from the juice of these canned peas. The organisms from the contents of the first cans incubated failed to show growth on plain nutrient and dextrose agar plates and deep dextrose shake cultures. A third can was opened and analyzed with an utmost care but the organisms again failed to grow. This revealed that in many previous analyses, both the coccus and bacillus organisms were readily and frequently stained from the contents analyzed, but the coccus form was only the organisms successfully isolated. A special media (pea agar) was used to grow these organisms which did not grow on plain media. A bacillus which was later identified as *Bacillus* "A", was successfully isolated from each

TABLE I.

Record of Spoiled Canned Peas Analyses.

Pea Samples in #	Ext. Cond. of cans:		Internal examination of Canned Peas.						
	Swell	Leaky	Gas	Full of Cont.	Color	Odor	Cond. of Liquid	Cond. of Peas.	Evid. of Etch.
#1.	H	?	+	N	N	N	N	N	-
#2.	S	+	?	3/4	N	N	N	S	+
#3.	S	?	?	N	P	B	C	S	+
#4.	H	?	+	N	N	P	C	S	-
#5.	H	-	+	N	N	N	C	N	+
#6.	H	?	+	N	C	P	C	M	?
#7.	H	-	+	N	N	N	C	M	-
#8.	S	-	-	N	N	N	N	N	-
#9.	H	+	+	N	P	N	C	M	+
#10.	H	+	+	P	B	P	C	S	+
#11.	H	+	+	N	P	P	C	M	+
#12.	S	-	+	N	N	N	C	N	-
#13.	H	-	+	N	N	N	C	N	-
#14.	H	-	+	N	P	P	M	S	+
#15.	S	+	?	3/4	P	P	M	S	+
#16.	H	?	+	N	N	N	N	N	-
#17.	H	-	+	N	N	P	C	S	?
#18.	H	-	+	N	N	N	C	N	-
#19.	H	-	+	N	B	P	C	M	+
#20.	S	?	?	N	P	P	C	S	+
#21.*	H	-	+	N	N	P	C	S	+
#22.*	H	-	+	N	N	P	C	S	?
#23.*	H	-	+	N	P	P	C	S	+
#24.*	H	-	+	N	N	P	M	S	+
#25.*	H	-	+	N	N	N	C	S	+
#26.*	H	-	+	N	N	P	C	S	+

Legend:

+ positive S soft
 - negative ? doubtful
 N normal M muddy
 H hard B bad
 * Swelled cans gathered from the same pack.
 D decomposed
 C cloudy
 P poor

TABLE I(Continued)

Record of Spoiled Canned Peas Analyses.

Pea Samples in #	Bact. Exam.		Cultures:			Type of Organisms isolated:
	Hanging drop.	Stained Prep.	Shake Cultures	Agar Plate	Dextrose Fern. Tube.	
#1.	-	+	+	+	-	Fac.Aner.Cocci
#2.	+	+	-	+	+	Yeasts & Molds
#3.	-	+	+	+	-	Fac.Aner.Cocci
#4.	-	+	-	+	-	Rods
#5.	-	+	+	+	-	Fac.Aner. Cocci
#6.	+	+	-	+	+	Yeasts & Rods
#7.	-	+	-	+	-	Rods
#8.	-	-	-	-	-	Apparently good
#9.	-	+	-	+	-	Rods, Cocci, Yeast
#10.	+	+	-	+	+	Yeasts, Molds, Rods
#11.	+	+	-	+	+	Yeasts, Rods
#12.	-	-	-	-	-	
#13.	-	+	+	-	-	Fac.Aner. Cocci
#14.	+	+	+	+	-	Rods & Cocci
#15.	+	+	-	+	+	Yeasts, Rods.
#16.	-	+	+	+	-	Fac.Aner. Cocci
#17.	-	+	-	+	-	Rods & Cocci
#18.	-	-	+	+	-	Fac.Aner.Cocci
#19.	+	+	-	+	-	Rods & Cocci.
#20.	-	+	-	+	-	Rods
#21.*	-	+	-	-	-	
#22.*	-	+	-	-	-	
#23.*	-	+	-	-	-	
#24.*	-	+	-	***	-	Rods**
#25.*	-	+	-	***	-	Rods**
#26.*	-	+	-	***	-	Rods**

Legend:

- + positive
 - negative
 N normal
 H hard
 * Swelled cans gathered from the same pack
 D decomposed.
 C cloudy
 P poor
 S soft
 ? doubtful
 M muddy
 B bad
 ** Rods isolated from the pea-agar plates only,
 but not in other media (Dextrose, Plain, etc)

of the remaining cans. This organism produces a vigorous growth on pea-agar plates even with loop dilutions up to the eighth plates. If, however, pure cultures were introduced to the plain nutrient and dextrose agar media, its growth was limited and very meager.

Of the organisms isolated during the present investigation, bacterial contamination was found to predominate in canned peas; molds and yeasts were present in six of the cans examined, while bacteria were found in twenty cases. Neither anaerobes nor thermophiles were isolated. The bacteria isolated were of two types; namely, the bacillus and the coccus. Three species of each type were morphologically and culturally described. Among the cocci are species which resemble the descriptions of *M. cereus* (Passet), *M. orbiculatus* (Wright), and *M. descidens* (Flugge). They are facultative anaerobes. The species of bacillus were identified as, B. "A", B. "B", and B. "C". They are strictly aerobic, and spore-formers. Descriptions are given as follows:

Organisms isolated and studied:

Bacillus A.

(Resembles *B. silvaticus*, Neide, morphologically and culture) (3)

1. Morphology:

(1) Vegetative Cells:

(a) Large rods, occur singly, in twos and in medium chains.

(b) Size- 2.34-3.90 x 0.55-0.78 microns.

(2) Endospores:

(a) Position- central, but no enlargement of rod on sporulation.

(3) Motility:

(a) Actively motile.

(4) Staining:

(a) Gram positive.

(b) Stains with ordinary dyes.

II. Cultural Characteristics:

(1) Nutrient Broth:

(a) Uniform cloudiness.

(b) Pellicle formed on the surface of the broth.

(2) Gelatin Colonies:

(a) Round, entire, regular, gelatin surface depressed.

(b) Size varies.

(c) Cream colored.

(d) Aerobic.

(3) Gelatin Stab:

- (a) Liquefaction- saccate.
- (b) Gelatin quickly liquefied, good growth, and flaky.

(4) Agar Slant:

- (a) Rhizoid, spreading, rather dry, slightly elevated and good growth.
- (b) Color- bright gray.

(5) Potato:

- (a) Growth- vigorous.
- (b) Color- dull wrinkled whitish gray, viscid and translucent.

(6) Milk:

- (a) Coagulated with solid curd.

(7) Litmus Milk:

- (a) Casein rapidly coagulated into a solid curd but not peptonized.

III. Physical Characteristics:

(1) Temperature relation:

- (a) Growth best at 37 degrees C.
- (b) Slow growth at 20 degrees C.
- (c) Not killed by 121.5 degrees C. for five minutes.
- (d) No growth when heated to 121.5 degrees C. for 15 minutes.

IV. Source- Soil.

Bacillus B.

1. Morphology:

(1) Vegetative Cells:

(a) Medium slender rods.

(b) Size- 2.6-4.0 x 0.46 microns.

(2) Endospores:

(a) Polar; very minute.

(3) Motility:

(a) Actively motile.

(4) Staining:

(a) Gram negative.

(b) Stain with ordinary dyes.

II. Cultural Characteristics:

(1) Nutrient Broth:

(a) Uniform cloudiness.

(2) Gelatin Colonies:

(a) Round, entire, small, regular.

(b) Color- dirty white, transparent, and
viscid.

(3) Gelatin Stab:

(a) Liquefaction- very slow, infundibuliform.

(b) In depth- very slow growth, filiform.

(4) Agar Slant:

(a) Growth- rather limited, smooth.

(b) Color- dirty white, translucent.

(5) Potato:

(a) No visible growth.

(6) Milk:

- (a) Not coagulated.
- (b) Slightly acid.

(7) Litmus milk:

- (a) Reduction- slightly acid.
- (b) Not coagulated.

III. Physical Characteristics:

(1) Temperature Relation:

- (a) Growth best at 37 degrees C.
- (b) No growth at 50 degrees C.
- (c) Slow faint growth at 20 degrees C.
- (d) Not killed by 121.5 degrees for five minutes.
- (e) No growth when heated to 121.5 degrees C. for 15 minutes.

Bacillus C.

I. Morphology:

(1) Vegetative Cells:

- (a) Very large rods, usually occur in twos.
- (b) Size- 1.72-4.68 x 0.52 microns.
- (c) One end rounded.

(2) Endospores:

- (a) Polar or capitate, large.
- (b) Size- 1.56 x 1.17 microns.

(3) Motility:

- (a) Motile.

(4) Staining:

- (a) Gram Positive.
- (b) Stain with ordinary dyes.

II. Cultural Characteristics:

(1) Nutrient Broth:

- (a) Uniform cloudiness.

(2) Gelatin Colonies:

- (a) Liquefiers, round, flaky, coarse, ring in center, entire, and granular.
- (b) Dirty white to dull gray.

(3) Gelatin Stab:

- (a) Liquefaction- stratiform, good growth, flaky, with heavy pellicles formed.

(4) Agar Slant:

- (a) Growth abundant, spreading, viscid, and smooth.
- (b) Color- dirty white turns to dull brown.

(5) Potato:

- (a) Growth abundant, viscid and smooth.
- (b) Color- dirty white.

(6) Milk:

- (a) Coagulated and peptonized.

(7) Litmus Milk:

- (a) Casein is rapidly coagulated. After about a week the casein is dissolved or peptonized.

III. Physical Characteristics:

(1) Temperature Relation:

- (a) Growth best at 37 degrees C.
- (b) No growth at 50 degrees C.
- (c) Good growth at 20 degrees C.
- (d) Good growth when heated to 100 degrees C. for 15 minutes.
- (e) Killed by 121.5 degrees C. for 5 minutes.

Micrococcus D.

(Resembles *M. cereus* Passet, morphologically and culturally with one exception, i. e. *M. cereus*, litmus milk unchanged) (3).

I. Morphology:

(1) Vegetative Cells:

- (a) Large cocci, one micron in diameter.
- (b) Occur in twos, tetrads, and in large clumps.

(2) Motility:

- (a) Non-motile.

(3) Staining:

- (a) Gram positive.
- (b) Stain with ordinary dyes.

II. Cultural Characteristics:

(1) Nutrient Broth:

- (a) Uniform cloudiness with dull yellowish sediment.

(2) Gelatin Colonies:

- (a) Very minute, like granules; microscopically

round to irregular, and reticulate.

(b) Yellowish color.

(3) Gelatin Stab:

(a) No liquefaction.

(b) Surface growth convex to pulvinate; color-
dull lemon-yellow.

(c) Depth- tuberculate with slight growth.

(4) Agar Slant:

(a) Bright greenish yellow turns to greenish
cream in two weeks.

(b) Growth fair, raised, and smooth.

(5) Potato:

(a) Growth moderate, viscid and smooth.

(b) Color- bright yellow, moist.

(6) Milk:

(a) Coagulated.

(b) Alkaline formed.

(7) Litmus Milk:

(a) Coagulated.

(b) Alkaline formed.

III. Physical Characteristics:

(1) Temperature Relation:

(a) Growth best at 37 degrees C.

(b) No growth at 50 degrees C.

(c) Slow growth at 20 degrees C.

(d) Fair growth when heated to 56 degrees C.

for ten minutes.

(e) No growth when heated to 58 degrees C.
for 10 minutes.

IV. Source: from pus.

Micrococcus E.

(Resembles *M. orbiculatus* Wright, morphologically
and culturally) (13)

I. Morphology:

(1) Vegetative Cells:

(a) Cocci minute, occur in twos, tetrads
and in small clumps.

(2) Motility:

(a) Non-motile.

(3) Staining:

(a) Gram positive.

(b) Stain with ordinary dyes.

II. Cultural Characteristics:

(1) Nutrient Broth:

(a) Uniform cloudiness.

(b) Sediment- flaky white with yellowish taint.

(2) Gelatin Colonies:

(a) Very minute, like granules; irregular;
margin undulated.

(b) Color- bright yellow.

(3) Gelatin Stab:

(a) No liquefaction.

(b) Surface- colonies flat, with orange color.

(c) In depth- medium tuberculate growth.

(4) Agar Slant:

(a) Growth- moderate.

(b) Color- Orange turns to brownish cream.

(5) Potato:

(a) Growth- moderate.

(b) Color- Orange.

(6) Milk:

(a) Not coagulated.

(7) Litmus milk:

(a) Not coagulated.

(b) Alkaline.

III. Physical Characteristics:

(1) Temperature Relation:

(a) Growth best at or from 20 to 37 degrees C.

(b) Not killed when heated to 55 degrees C for 10 minutes.

(c) No growth when heated to 57 degrees C. for 10 minutes.

IV. Source: from water.

Micrococcus F.

(Resembles *M. descidens* Flugge, morphologically and culturally) (13)

I. Morphology:

(1) Vegetative Cells:

- (a) Cocci minute, occur singly, in twos and in tetrads.

(2) Motility:

- (a) Non-motile.

(3) Staining:

- (a) Gram positive.
- (b) Stain with ordinary dyes.

II. Cultural Characteristics:

(1) Nutrient Broth:

- (a) Uniform cloudiness.

(2) Gelatin Colonies:

- (a) Very minute, like granules; round, entire, and moruloid.

(3) Gelatin Stab:

- (a) Liquefaction- cratiform.
- (b) In depth- filiform with slight growth.

(4) Agar Slant:

- (a) Growth- moderate, smooth, viscid.
- (b) Color- dull yellow turns to creamy yellow in two weeks.

(5) Potato:

- (a) Growth- moderate, moist, spreading.
- (b) Color- orange-yellow.

(6) Milk:

(a) Coagulated forming a solid curd.

(7) Litmus Milk:

(a) Coagulated.

(b) Slightly acid.

III. Physical Characteristics:

(1) Temperature Relation:

(a) Growth best in temperature between
20 to 37 degrees C.

(b) Not killed when heated to 55 degrees
C. for 10 minutes.

(c) No growth when heated to 57 degrees
C. for 10 minutes.

IV. Source:

(1) From air or water.

Explanations to Account for the Presence of the
Coccus-forming Organisms in Apparently tight-
ly Sealed Canned Food.

In this experiment the coccus-forming organisms were isolated from nearly forty percent of all cans analyzed. In eight cases, micrococci were found in cans that are apparently tightly sealed while in others they are present in cans showing evident leakage. Close observations from the works done by other co-workers of the Advanced Zymology and Fermentation class and from examinations made by the students of the Canning classes, strengthen the belief that the bacteria of this genus were invariably found present in a large percentage of the spoiled or suspected canned foods. Since these organisms are non-spore formers, the possible explanations for their presence in the canned foods may possibly be accounted for in the following ways:- First, they gain entrance through tiny or minute openings of defective cans--such openings may or may not be detected by the naked eye. Many cans used in the canning industry may be put up in such a state that is considered as perfect, but the presence of a few microscopic openings is sufficient to make possible the entrance of bacteria into the cans. Their admittance is hastened during the cooling process of the foods canned--as the foods are exhausted and processed there is developed a partial vacuum in the

cans, if microscopic openings should be present in the cans while the cans are being cooled, certain amount of air accompanied with bacterial organisms from without will rush in and take the place of the partial vacuum until these microscopic openings are finally sealed by the particles in the content itself. Whenever such condition happens it will not only permit the entrance of many organisms, but also furnish oxygen for the aerobes to develop within the apparently sealed cans. Secondly, the non-spore forming organisms, such as the coccus, present in the canned foods may be due to underprocessing. Bovie and Bronfenbreuner (21) describe an apparatus employing thermo-couples for measuring the rate of heat penetration in tin cans of navy beans. Their result discloses that although a constant autoclave temperature of 280 degrees F. was reached in 15 minutes, the center of the can did not approximate this temperature for much more than 15 minutes during the last of the 180 minute processing period. Thus it shows that a difficulty of securing a uniform temperature throughout the entire content of the can during the period of processing may account for the non-spore forming organisms found present in great many cases. Lastly, it is not uncommon to find that oftentimes the cans pass through the exhausted box and are delayed in processing due to a temporary breaking down of the machine. As the latter is being repaired, the contents of the cans are become cooled, and if these delayed cans

are later processed along with others that are just passed out through exhausted box while still hot, and using the same length of time in all cases, therefore, a chance of killing the organisms present will be greatly decreased.

Determination of the Thermal Death Point:

In the determination of the "Thermal Death Point" on organisms described, the general directions outlined by Frost (19) were closely followed. In order to obtain a best possible result, two things need to be taken into consideration, namely, first a bouillon culture of each organism to be determined must be reasonably young--preferably, a forty-eight hours culture. Young cultures withstand a higher temperature than the older ones. Secondly, the time and temperature employed during each determination must be as constant as possible--a slight variation in temperature or in the shortening or lengthening the time of heating will invariably affect the final result.

TABLE II.

Table on Thermal Death Point Determination.

Organisms	45°C.	50°C.	55°C.	56°C.	57°C.	58°C.	59°C.	60°C.	80°C.	100°C.	Autocl. Pr. 15#		Results.
											5"	15"	
B. "A"	+	+	+	+	+	+	+	+	+	+	+	-	
B. "B"	+	+	+	+	+	+	+	+	+	+	+	-	
B. "C"	+	+	+	+	+	+	+	+	+	+	+	-	
C. "D"	+	+	+	+	-	-	-	-	-	-	-	-	T.D.P. 57°C.
C. "E"	+	+	+	-	-	-	-	-	-	-	-	-	T.D.P. 56°C.
C. "F"	+	+	+	-	-	-	-	-	-	-	-	-	T.D.P. 56°C.

+ growth.

- growth stopped.

T.D.P. Thermal Death Point.

B. bacillus.

C. coccus.

"A"-----"F", strains of different organisms.

+ slight growth.

-

The results obtained from this experiment are found tabulated in Table II. All species of bacillus described are spore-formers, and therefore they are very resistant to heat. Whilst the species of coccus identified will not survive heating to 57 degrees C. for 10 minutes.

Gas Fermentation:

Attempts have been made to determine from the organisms described whether or not these bacillus-formers will produce gas individually, if not, would they produce gas according to explanations of Sear and Putnan (24)

namely, the gaseous fermentation by two organisms in symbiosis of a substance from which neither organism acting alone can produce gas? Various fermentation media were employed. Results found are tabulated in Table III.

TABLE III.

Record of Gas Fermentation Determination.

Organisms acted alone or in combination of:	Gas Formation from:	
	Lactose.	Pea juice.
(resemble to B."A". B. silvaticus)	-	2cc.
B."B".	-	-
B."C".	-	-
B."A". + B."B".	-	1½cc.
B."A". + B."C".	-	1½cc.
B."B". + B."C".	-	2cc.
B."A". + M. cereus.	-	1.5cc.
B."A". + M. orbiculatus.	-	1.5cc.
B."A". + M. descidens.	-	1.5cc.
B."B". + M. cereus.	-	-
B."B". + M. orbiculatus.	-	-
B."B". + M. descidens.	-	1.5cc.
B."C". + M. cereus.	-	-
B."C". + M. orbiculatus.	-	-
B."C". + M. descidens.	-	1.5cc.

The experiment disclosed that gas formation was procured from pea media but none from the lactose media. This has been previously accounted for that organisms described will act favorably only when certain food substances are present in a medium. From the pea media, gas formation was evident in nine tubes as shown in Table III. In one of the tubes, gas was produced by the action of a bacillus alone, while in others by two organisms in symbiosis growing in a medium from which neither organisms acting alone can produce gas. Further demonstration by inoculating pure cultures into sterile commercial canned peas corroborated the previous findings.

Summary:

1. Organisms isolated being held responsible for the spoilage in canned peas besides yeasts and molds from the leaky cans are bacteria, 20 cultures representing 2 genera of 3 species each.

2. All bacilli found are spore-formers.

3. Coccus-formers isolated and identified are *M. cereus*, *M. orbiculatus*, and *M. descidens*. They are facultative anaerobes.

4. Neither anaerobes nor thermophiles were found present in canned peas studied.

5. Organisms described cannot produce gas when they are acting alone, ^{but one. However,} ~~but~~ there are evidences of gaseous fer-

mentation when two organisms are acting under symbiotic condition.

6. The "Thermal Death Point" of all spore-forming bacilli found is comparatively high. They are not killed when heated in autoclave with 15 pound pressure for 5 minutes.

Conclusion:

The spoilage in canned peas is largely due to the action of organisms present in the contents of the cans. They consist of both aerobes and facultative anaerobes. Their presence may be accounted for either that foods have been underprocessed, or that they have been delayed in processing due to the stopping of machinery, or both. Although the use of defective cans in canning is questionable, because of a large percentage of the spoiled or suspected canned peas studied containing non-spore forming organisms. Therefore, before canning is anticipated, it is necessary to pay more attention to the processing and to the conditions of the cans used.

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